

FIG.1A

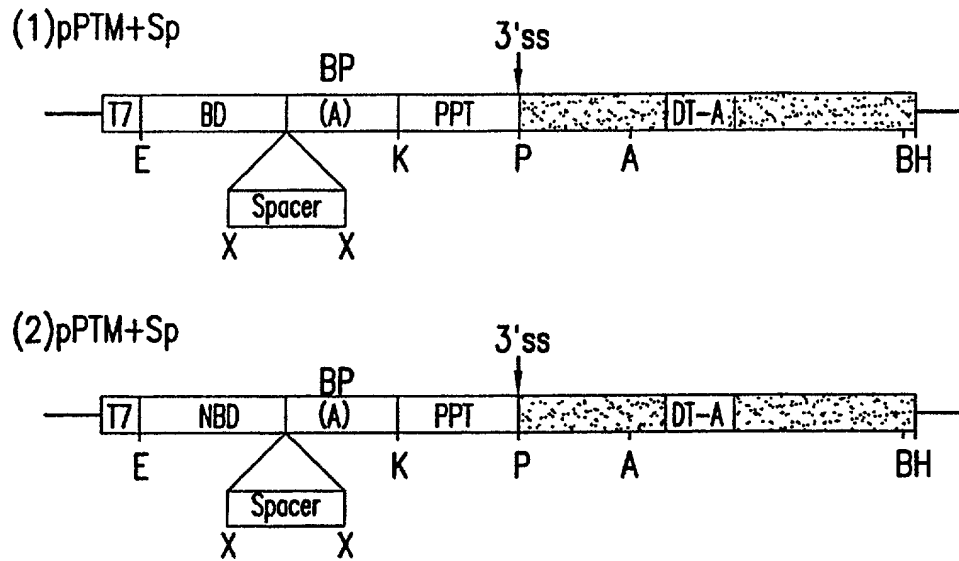


FIG.1B

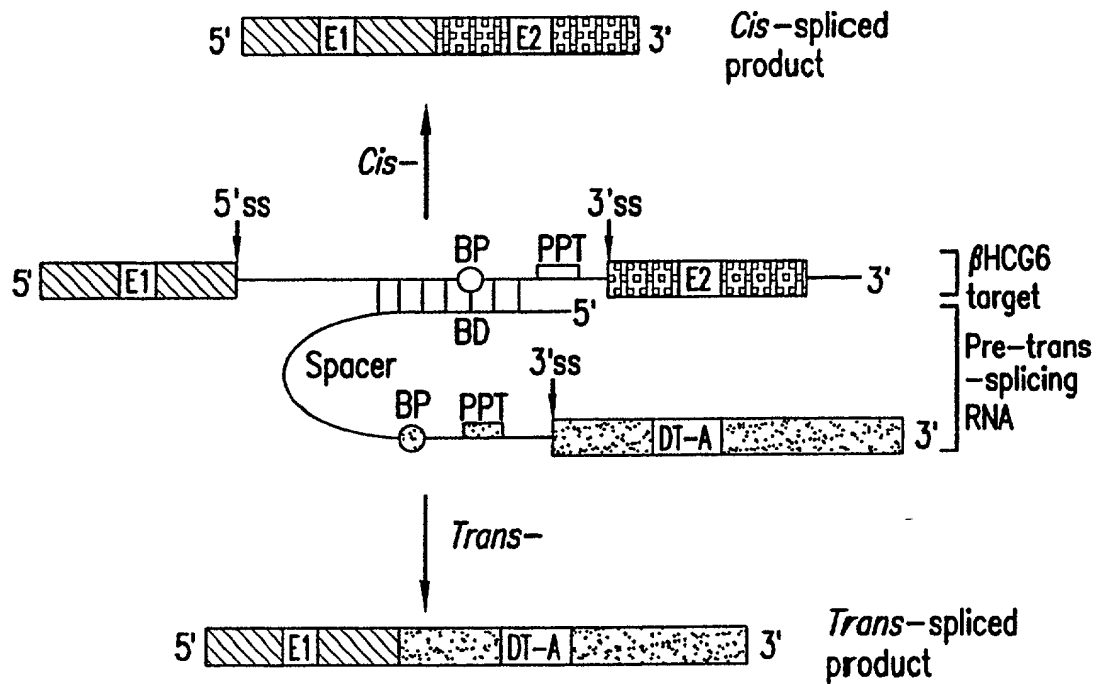


FIG.1C

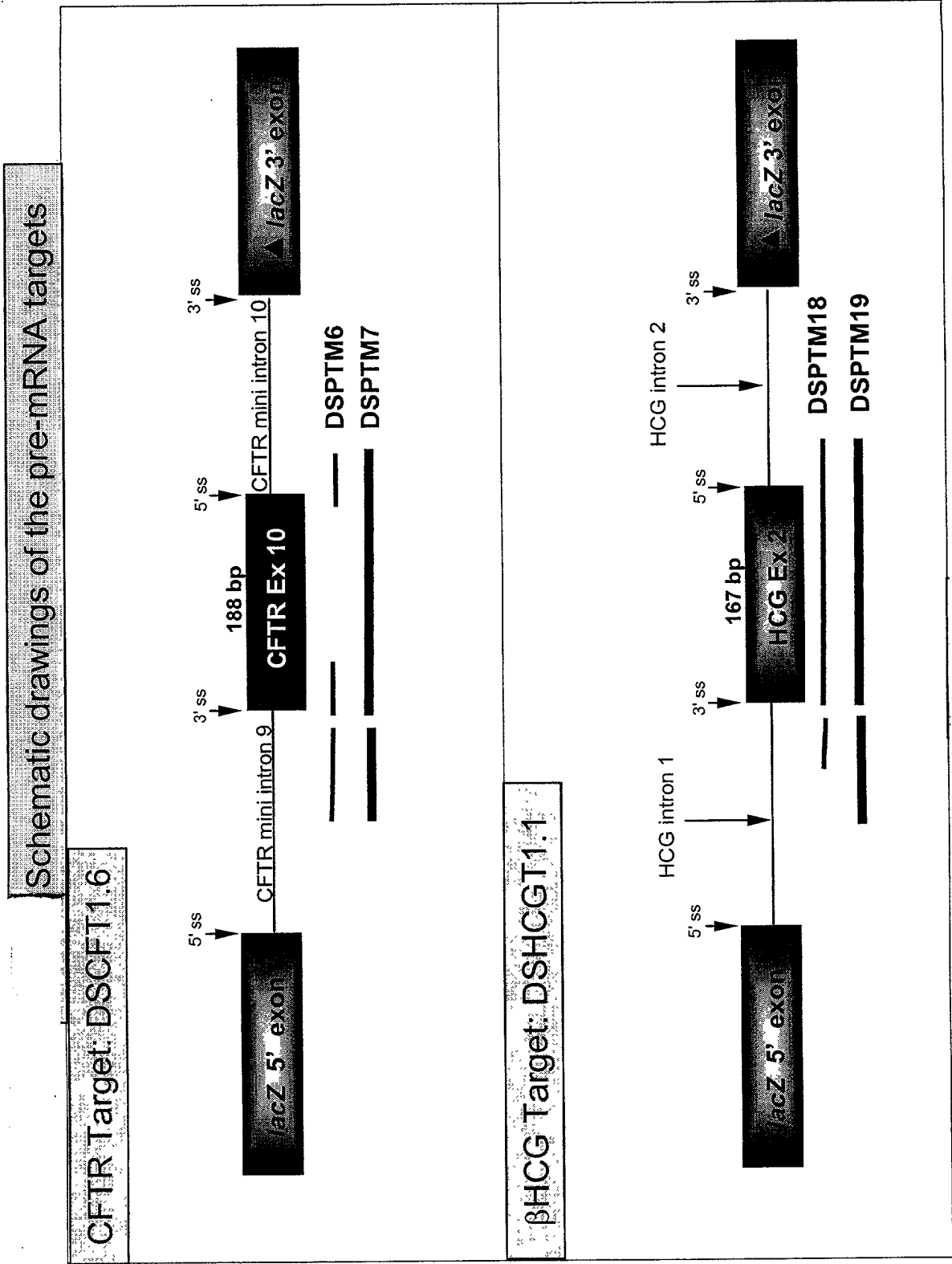
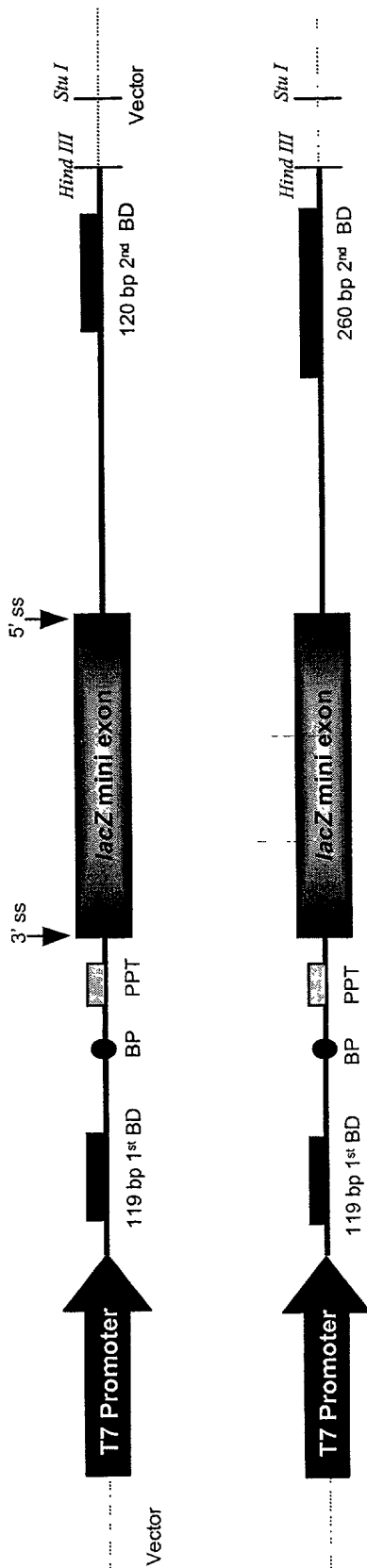


Figure 2

Schematic diagrams of double trans-splicing PTMs

DSPTM6 & 7 (CFTR Targeted)



DSPTM18 & 19 (βHCG Targeted)

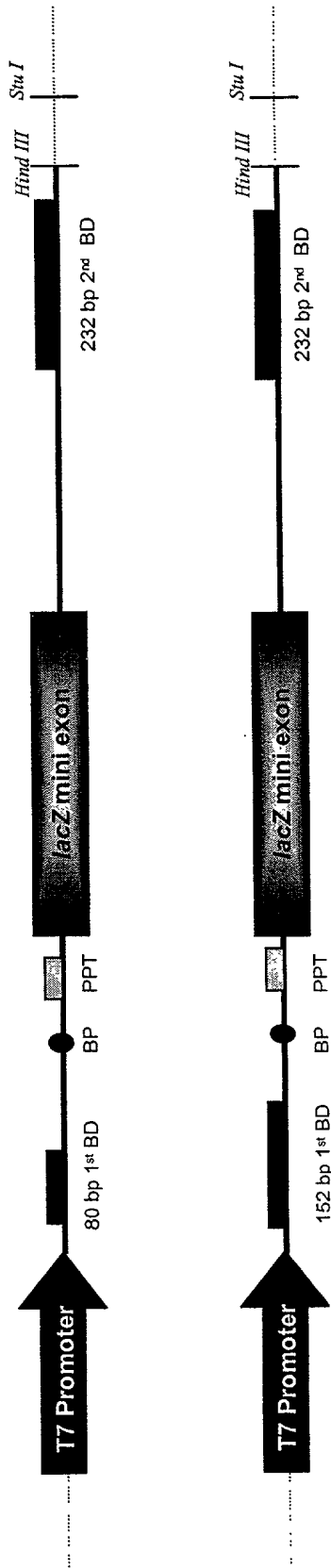
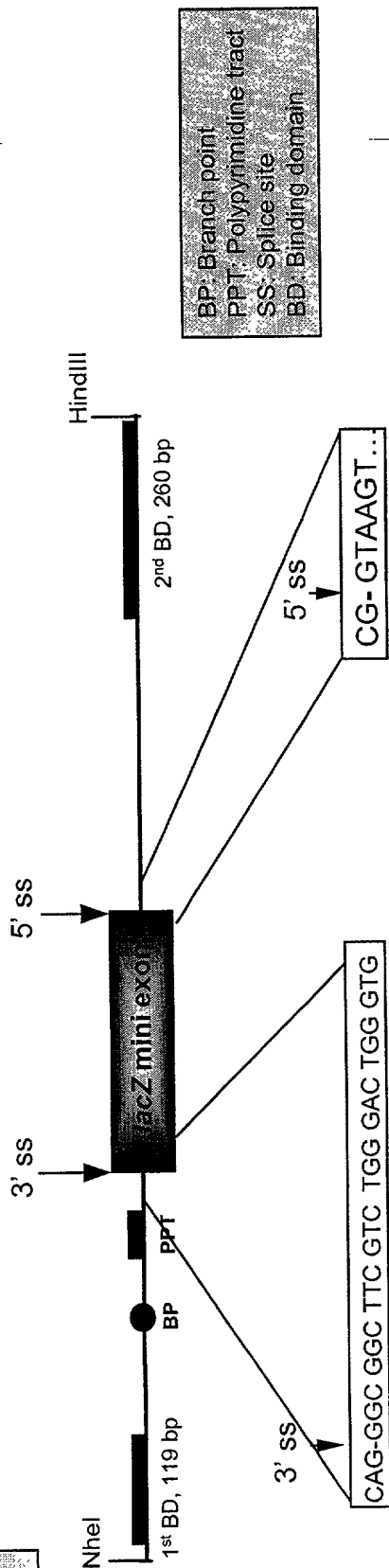


Figure 3

Diagram and important structural elements of double trans-splicing PTM7

DSPTM-7



1st BD (119 bp) : GATTCACCTTGCTCCAAATTATCATCCTAAGCAGAAAGTGATATATTCTTATTGTAAAGATTCTATTAACCTCATTGTGATTCAAAATA
TTTAAATACTTCCTGTTTCATACTCTGCTATGCAC

Spacer sequences: AACATTATTATAACGTTGCTCGAA

BP, PPT and acceptor splice site: TACTAAC T GGTACC TCTTCTTTTTTTTTT GATATC CTGCAG GGC GGC TTC GTC TGG GAC TGG
BP PPT 3' ss lacZ mini exon

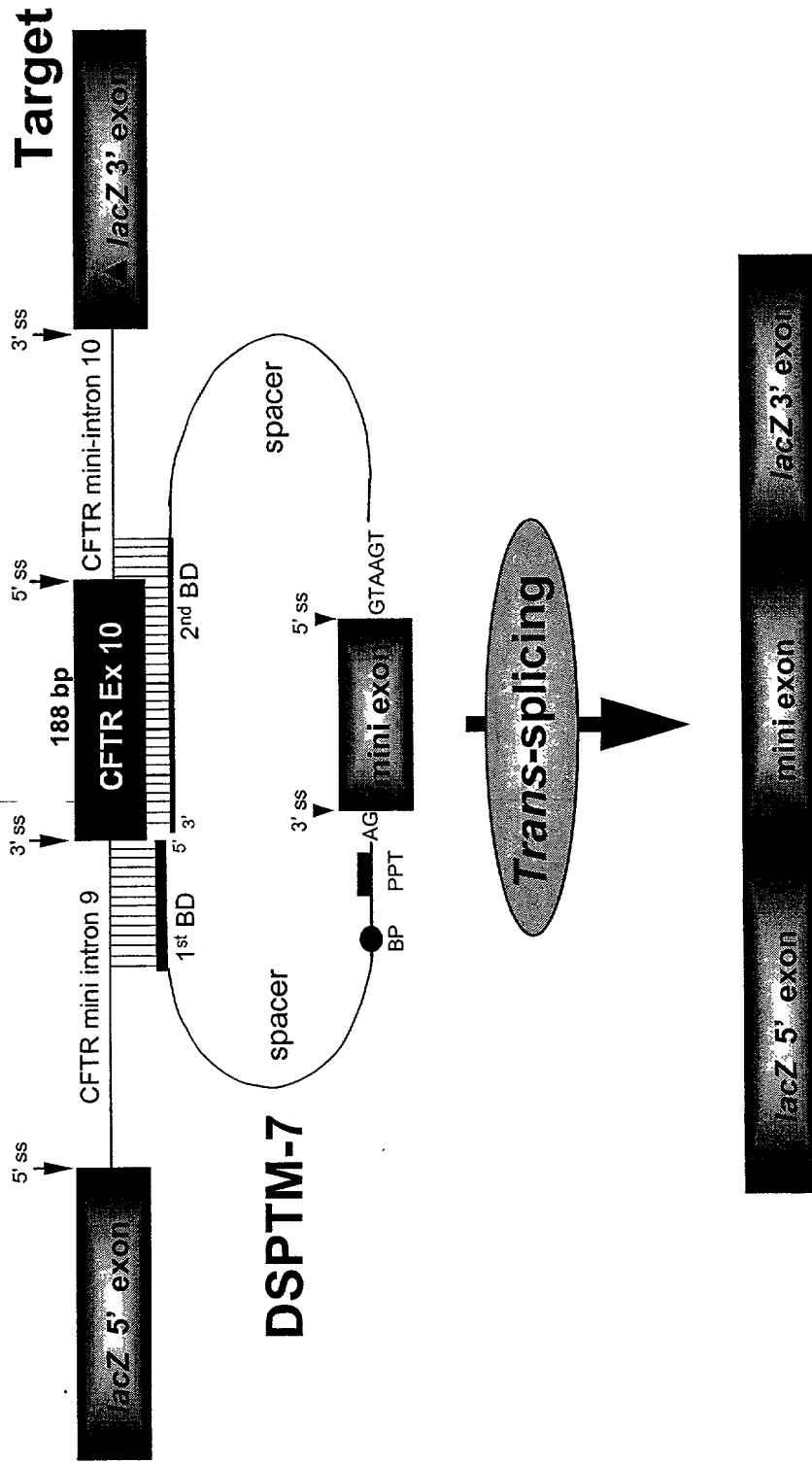
5' donor site and 2nd spacer sequence: IGA ACG GTAAGT GTTATCACCAGATATGTCTAACCTGATTCGGGCCCTTCGATACGCTAA
GATCCACCGG

2nd BD (260 bp): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAAATTCATGCTTTGATGACGCTTCTGTATCTATTCATTCATTGGAA
ACACCAATGATTTTCTTTAATGGTCCCTGGCATAATCCTGGAAACTGATAACACAATGAAATCTTCCACTGTGCTTAA
AAAAACCCCTCTGAAATTCCTCCATTCTCCATAATCATCATTAACAACCTGAACTCTGAAATAAAACCCATCATTATTAACTCA
TTATCAAATCACCG

Figure 4

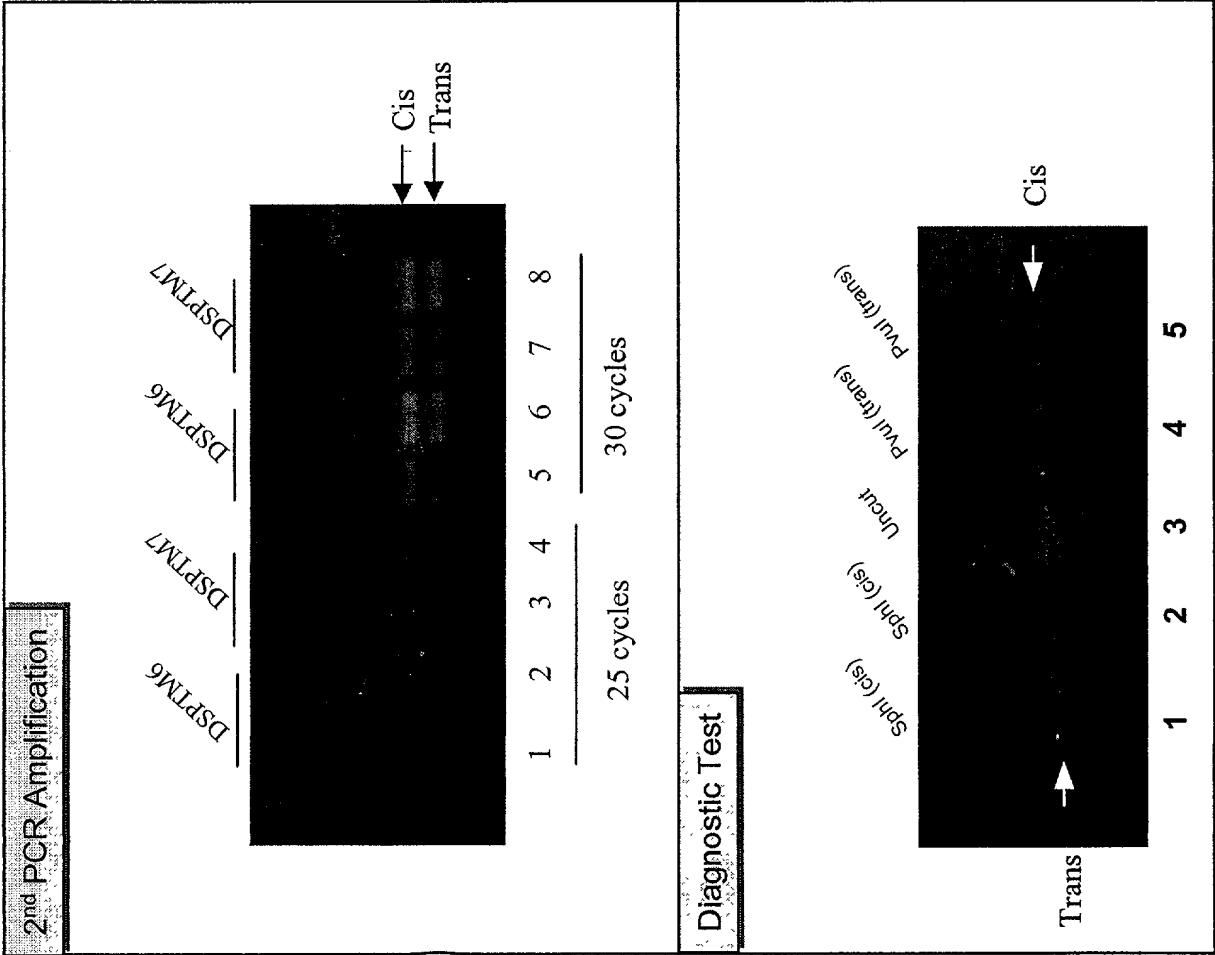
Figure 5

Double trans-splicing β -gal repair model



Accurate double *trans*-splicing between the target pre-mRNA and synthetic PTM RNA will result in the production of repaired *lacZ* mRNA

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells



DSPTM6 and 7 (CFTR targeted)

Methods

Transfect 293T cells with DSPTM6 and DSPTM7 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycles, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F+Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp.

Diagnostic test: Digest PCR product with *Pvu* I (*trans*-specific) and with *Sph* I (*cis*-specific) at 37°C for 2-3 hr

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6A

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in stable cells

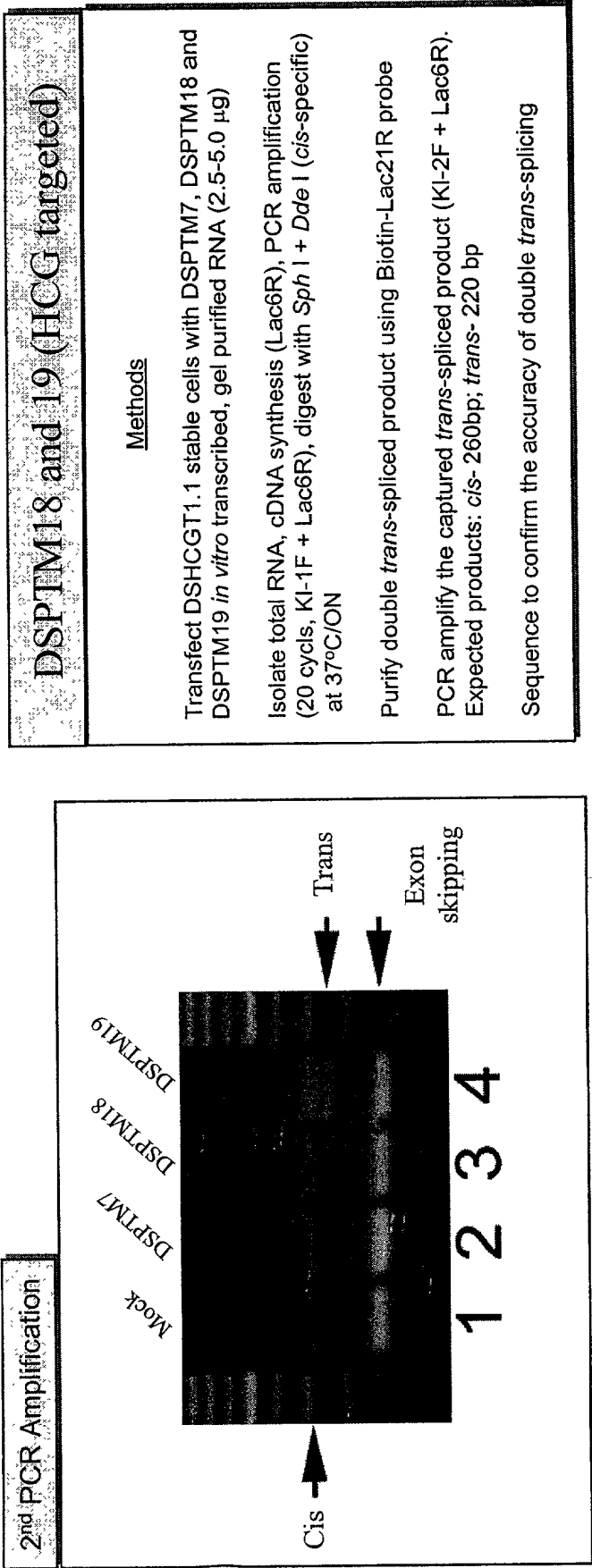


Figure 6B

Accuracy of double *trans*-splicing of synthetic PTM RNA in 293T cells

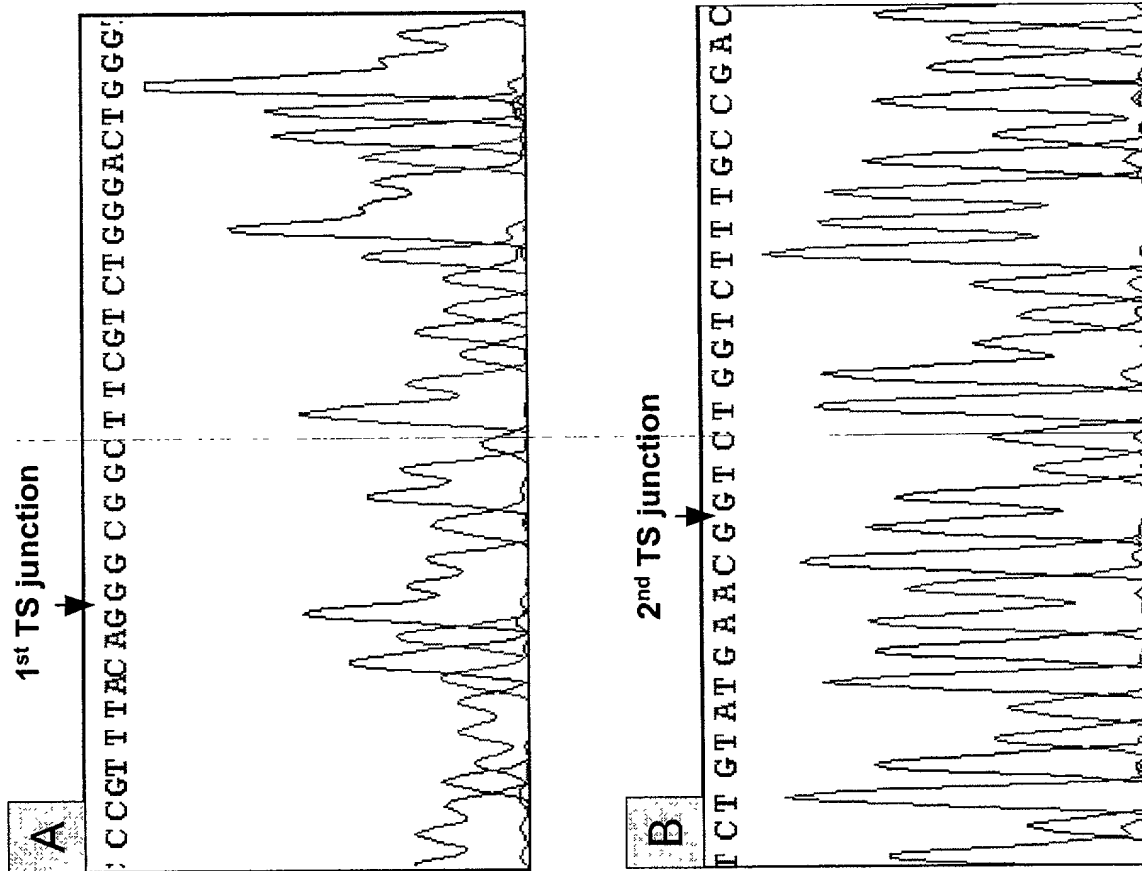


Figure 6C

Restoration of β -gal function through RNA transfection in 293T cells
(Proof-of-concept for SMaRT RNA Therapeutics!!)
Synthetic RNA, Double trans-splicing

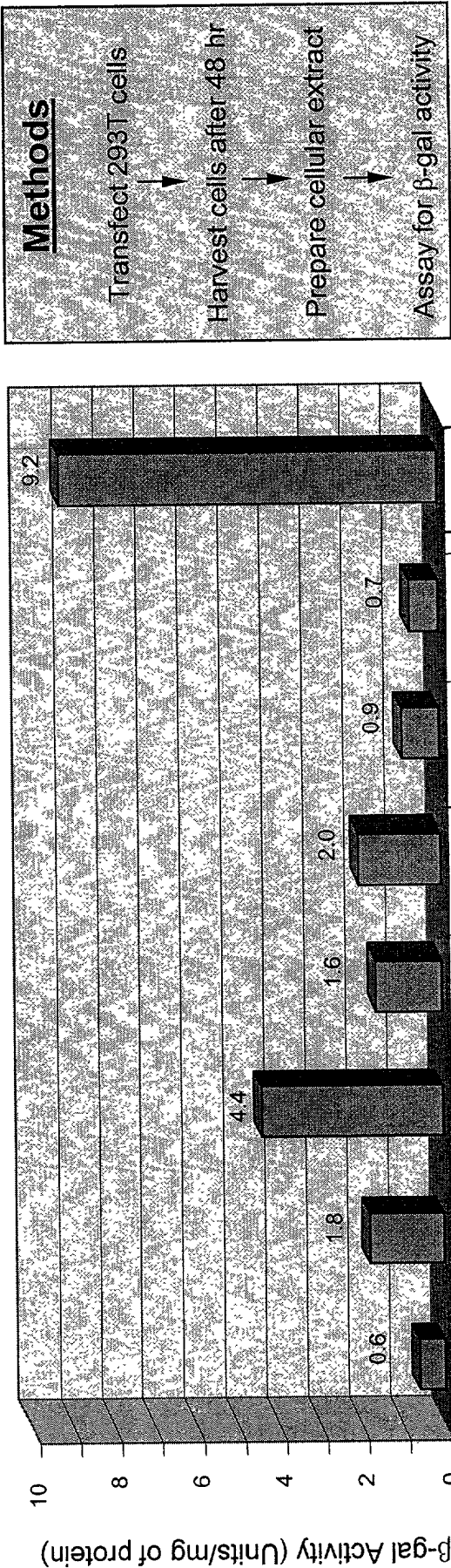


Figure 7